including arrhythmia and heart failure. Acute doxorubicin cardiotoxicity is multifaceted and includes the formation of reactive oxygen species, apoptosis, and disturbances in calcium handling within the intracellular sarcoplasmic reticulum (SR) store. Our focus is on the protein targets within the SR that bind doxorubicin, namely the ryanodine receptor (RyR2) calcium release channel and the calcium binding protein, calsequestrin (CSQ2) which also regulates RyR2, and how drug binding disturbs SR calcium homeostasis. Such disturbances are known to cause cardiac dysfunction. Our previous *in vitro* assays showed that doxorubicin reduced CSQ2's calcium binding capacity, altered SR calcium homeostasis and impaired RyR2 activity, as a consequence of the drug binding directly to these proteins and to drug-induced RyR2 oxidation.
To investigate the *in vivo* modifications of RyR2 and other SR proteins by doxorubicin, we developed an acute mouse model of doxorubicin cardiotoxicity. Doxorubicin treatment reduced expression of RyR2, the SR calcium pump SERCA and CSQ2 and lowered the fraction of CSQ2 associated with RyR2. The decline in SERCA and RyR2 expression and the reduced proportion of RyR2 that can be activated by CSQ2 may underlie the significantly perturbed SR calcium release after doxorubicin treatment. In addition, doxorubicin treatment caused "hyper" phosphorylation of RyR2 at S2808 and significant dissociation of the RyR2 dephosphorylating enzymes PP1 and PP2A. Hyperphosphorylation of S2808 is known to cause RyR2 dysfunction, afterdepolarizations, arrhythmia and heart failure. It is likely other modifications in RyR2 function (oxidation, ligand-binding) in addition to RyR2 hyperphosphorylation and reduced CSQ association underlie the multi-faceted disturbances in calcium release that contribute to the clinical cardiotoxicity induced by doxorubicin treatment.

2815-Pos Board B585
Ros and CaMKII Modulate Sodium Overload-Induced Calcium Leak from Ryanodine Receptors in Ventricular Myocytes
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Introduction: Elevation of the diastolic free calcium concentration ($[Ca^{2+}]_{cyt}$) alters excitation-contraction coupling and contractility in cardiac myocytes. It is known that Na^+ overload increases the levels of diastolic $[Ca^{2+}]_{cyt}$ and induces arrhythmias via reverse mode of Na^+/Ca^{2+} exchanger. We hypothesized that Ca^{2+} leak induced by oxidation/phosphorylation of ryanodine receptors (RyRs) contributes to the rise in $[Ca^{2+}]_{cyt}$ during Na^+ overload.
Methods: We used confocal fluorescence imaging of intact and membrane-permeabilized isolated rabbit and rat ventricular myocytes to study Ca^{2+} transients, cytosolic sodium ($[Na^+]_{cyt}$) and intracellular reactive oxygen species (ROS) levels. Cellular Na^+ overload was induced using 5 nM anemone toxin-II (ATX-II), a late Na^+ current (I_{NaL}) enhancer.
Results: ATX-II-induced $[Na^+]_{cyt}$ overload caused abnormal Ca^{2+} transients in intact rabbit and rat myocytes. Additionally, high cytosolic Na^+ increased ROS levels and activated CaMKII. These effects were abolished by the INa inhibition with ranolazine and TTX, CaMKII inhibition with KN93 and AIP or by the application of antioxidants such as DTT and coenzyme Q10. In the experiments with membrane-permeabilized myocytes, rapid elevation of $[Na^+]_{cyt}$ from 5 mM to 20 mM resulted in an increase of ROS production in mitochondria and Ca^{2+} leak from RyRs by measuring the frequency of spontaneous Ca^{2+} waves. The CaMKII inhibitors, KN-93 and AIP, attenuated the effects of high $[Na^+]_{cyt}$ on Ca^{2+} signaling.
Conclusions: These data suggest that $[Na^+]_{cyt}$ regulates RyRs activity. An increase in $[Na^+]_{cyt}$ induces ROS production; ROS activates CaMKII leading to altered Ca^{2+} transients and abnormal Ca^{2+} handling.

2816-Pos Board B586
Hyperaldosteronism Severely Effects Calcium Handling, Contractility and Action Potentials in Isolated Cardiomyocytes of Rats
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Evidence is increasing that aldosterone is not only regulating renal salt reabsorption but might also be a key player in the development of cardiomyopathies. Especially, chronically elevated levels of aldosterone (hyperaldosteronism) participate in the generation of atrial fibrillation, tachycardia, hypertrophy, fibrosis or inflammation. We elevated blood plasma levels of adult rats by implanting osmotic mini-pumps in 8 week old rats for 8 weeks (1,5 µg/h aldosterone). For the exploration on cellular excitation and contraction we performed isolation of ventricular and left and right atrial myocytes and studied cellular calcium handling, contractility and electrical properties of the cells.
In ventricular myocytes we observed a severe drop-down of resting calcium, 1st amplitude (A1st) and steady-state amplitude (Astst) of global electrically induced calcium transients. For atrial myocytes of the right atrium such changes were rather pronounced while surprisingly, the left atrial myocytes displayed such effects to a much lesser degree. In line with changes in calcium handling contractility of the myocytes was also impaired. Here, right atrial myocytes displayed a pronounced negative contractility-frequency relationship (0.2 - 2 Hz) and their resting/diastolic sarcomer length was massively increased when compared to cells from control animals. When patch clamping the atrial myocytes we also found substantial changes in their action potentials (AP). Interestingly, for both left and right atrial cells, early phases of repolarisation were massively shortened (>50% change). Right atrial cells also showed a reduced AP rise time and their capacitance were significantly reduced (>30%).
Here we demonstrate for the first time a differential effect of hyperaldosteronism on ventricular myocytes and cells from the left and right atrium.
This work was supported by the DFG, BMBF and the Medical Faculty.

2817-Pos Board B587
Regulatory Role of Histidine Rich Calcium Binding Protein (HRC) in Cardiac Sarcoplasmic Reticulum Ca2+ Release Complex
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Ca2+ release from sarcoplasmic reticulum (SR) in cardiac muscle is mediated by a multiprotein complex that includes the RyR, triadin, calsequestrin (CSQ), junctin and HRC. The interaction of these proteins with each other, the luminal SR Ca2+ concentration and the influx of Ca2+ from the L-type Ca2+ channel (LTCC) determines the activation of RyR. Triadin has a charged luminal region which interacts with Ca2+ binding proteins such as CSQ, HRC and thus can directly or indirectly regulate RyR activity. In the present study using an in vitro binding assay and deletion mutants, we found that only the distal region of triadin interacts with RyR, CSQ and HRC and that the 4th KEKE motif (amino acid 202-231) of triadin is the minimal interacting region to interact with all the three proteins. We compared the interaction of triadin with the individual proteins by co-immuno precipitation of triadin interacting partners at different levels of free Ca2+ concentrations. The result showed maximal RyR interaction with triadin at pCa 3, HRC at pCa 4 and, CSQ at pCa 7 and 3. To study whether these proteins compete with each other to interact with triadin we conducted a competitive binding assay. The results showed no competition between RyR (loop II) and CSQ, while a significant competition was noticed between HRC and RyR (loop II) to bind to triadin. Taken together, we suggest that HRC interacts with triadin at intermediate luminal Ca2+ concentration and has an important role in maintaining the refractoriness of Ca2+ release by decreasing RyR activity. (Supported by Korea MEST NRF grant (2011-0002144), the 2011 GIST Systems Biology Infrastructure Establishment Grant and KISTI-KREONET).

2818-Pos Board B588
Severe Cardiac Hypertrophy and Depressed Contractility in Transgenic Mice with Co-Overexpression of Calsequestrin and SERCA2a
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Cardiac-specific overexpression of calsequestrin (CSQ) is associated with hypertrophy, depressed contractility, impaired SR Ca^{2+} release and signs of heart failure. The Ca^{2+}ATPase (SERCA2a) represents the major determinant of myocardial contractility. Its stable or transient expression results in increased contractile parameters and higher Ca^{2+} transients. Hence, we tested whether the additional expression of SERCA2a in CSQ-overexpressing hearts results in beneficial functional effects. For this purpose, we generated double transgenic mice overexpressing both CSQ and SERCA2a (TG^{CxS}) by cross-breeding of single transgenic mouse lines (TG^{CSQ} and TG^{SER}). SERCA2a protein expression was increased by 1.4-fold in both TG^{SER} and TG^{CxS} compared to wild-type (WT) hearts. Consistently, at submaximal free Ca^{2+} levels (30 nmoL/L), $^{45}Ca^{2+}$ uptake reached 12%, 25%, 14% and 30% of maximal Ca^{2+} levels in WT, TG^{SER}, TG^{CSQ} and TG^{CxS}, respectively (n=5, $P<0.05$). CSQ was overexpressed by 10-fold in both TG^{CSQ} and TG^{CxS} compared to WT. The expression of triadin was decreased by 53% in both TG^{CSQ} and TG^{CxS} compared to WT ($P<0.05$). The ryanodine receptor expression was reduced by 44%, 33% and 86% in TG^{CSQ}, TG^{SER} and TG^{CxS}, respectively, compared to WT ($P<0.05$). In addition, co-overexpression of CSQ and SERCA2a was

associated with severe cardiac hypertrophy. We observed a heart weight to body weight ratio of 4.4 ± 0.1, 4.9 ± 0.1, 8.9 ± 0.3 and 12.5 ± 1.0 mg/g in WT, TG^{SER}, TG^{CSQ} and TG^{CxS}, respectively (n=7, $P<0.05$). Stained tissue sections revealed an enhanced interstitial fibrosis in ventricles of both TG^{CSQ} and TG^{CxS}. Functional analysis was performed on isolated working heart preparations. Contractility (+dP/dt) was depressed in TG^{CxS} (1378 ± 144 mmHg/s) compared to WT, TG^{SER} and TG^{CSQ} (2461 ± 119, 3644 ± 161 and 1879 ± 105 mmHg/s, respectively, n=6, $P<0.05$). We conclude that the additional overexpression of SERCA2a failed to rescue the impaired cardiac phenotype of CSQ transgenic mice.

2819-Pos Board B589

Ablation of Histidine-Rich Calcium Binding Protein (HRC) Results in Severe Cardiac Hypertrophy and Dysfunction in Pressure Overload Model

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HRC is a high capacity Ca^{2+} binding protein located in the lumen of sarcoplasmic reticulum (SR). Previously, we had shown that HRC knock-down not only led to enhanced Ca^{2+} release and Ca^{2+} uptake in SR, but exacerbated cardiac function after transverse aortic constriction (TAC), suggesting its important role in Ca^{2+} cycling and cardiac function. HRC-KO mice were generated and used for the present study. Pressure overload induced by TAC in the KO mice heart resulted in severe cardiac hypertrophy with 27% increase of heart weight (HW) per body weight (BW) ratio and 25% increase of tibia length per BW ratio as compared to WT TAC mice. HRC KO mice also showed decreased fractional shortening (FS) by 37%, increased TGF-β expression by 2 folds, severe cardiac fibrosis and highly increased number of TUNEL positive signals in heart tissue compared to WT TAC mice. The electrocardiogram (ECG) study showed shorter RR interval, faster heart rate and decreased R amplitude in HRC KO TAC mice. The incidence of arrhythmia was significantly increased in HRC KO mice after **intraperitoneal injection of caffeine (120 mg/kg BW) and epinephrine (2 mg/kg BW),indicating that HRC KO mice are more susceptible to epinephrine and caffeine injection, consistent with the previous report (**Jaehnig **et al.** Mol. Cell. Biol.**, 2006).** The survival rate of HRC KO mice was significantly decreased after TAC. Taken together, our results suggest that ablation of HRC could lead to altered SR Ca^{2+} cycling and deterioration of cardiac function under pathological condition. (Supported by Korea MEST NRF Grant (20110002144), the 2011 GIST System Biology Infrastructure Establishment Grant and KISTI-KREONET).

Cardiac Muscle III

2820-Pos Board B590

Myofilament Phosphorylation and Function in Diastolic Heart Failure

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This study aimed to compare alterations in phosphorylation and function of sarcomeric proteins in diastolic heart failure (DHF) vs. normal myocardium under two conditions of tissue procurement: beating-heart biopsy and postmortem tissue sampling. Left ventricular tissue samples were procured from normal (CTRL) or old dogs made hypertensive by renal wrapping (DHF), by either taking biopsies of the beating heart (n=7) or excising tissue postmortem (n=8). Isolated permeabilized cardiomyocytes were attached to a force transducer and passive tension ($F_{passive}$) was measured between 1.8 and 2.4 μm, calcium sensitivity (pCa_{50}) at 2.2 μm sarcomere length. Phosphorylation of myofilament proteins was assessed using the SYPRO-Ruby (total protein) / Pro-Q Diamond (phosphoprotein) system. Expression of total or phosphorylated protein, including titin-PEVK, cTnI, and PKCα was also quantified by immunoblot. Postmortem tissues and biopsy samples showed similar changes in DHF vs. CTRL, including increased phospho-PEVK, phospho-PKCα, and $F_{passive}$, but decreased titin N2BA:N2B isoform ratio and reduced phosphorylation of total titin, cMyBPC, cTnT, cTnI and cTnI (S23/24). Differences were apparent in terms of lowered pCa_{50} in postmortem DHF, but increased pCa_{50} in biopsy DHF. Reduced MLC2 phosphorylation in DHF vs. CTRL was observed in biopsies, but not in postmortem tissues. A degradation form of titin (T2) was more abundant in postmortem tissues than in biopsies. We conclude that this DHF model is characterized by titin-isoform shift towards the stiff N2B isoform, a deficit in total titin phosphorylation, but increases in PKCα-dependent phosphorylation of titin-PEVK-domain and cardiomyocyte $F_{passive}$, as well as altered pCa_{50}. For a few parameters, postmortem and beating-heart samples differed in the direction of change in DHF vs. CTRL, particularly regarding Ca^{2+}-sensitivity. However, most parameters changed in the same direction, suggesting the cellular events associated with death alter sample properties to a modest degree.

2821-Pos Board B591

Troponin Phosphorylation Crosstalk

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The troponin (Tn) complex is a critical regulatory and central integrative hub for post-translational modifications (PTM) within the thin filament. There are a number of Tn phosphorylations; however, β-adrenergic-induced protein kinase A (PKA) phosphorylation of cardiac troponin I (TnI) acts as the major sarcomeric modulator of function. Traditionally, individual phosphorylation sites have been studied, yet simultaneous phosphorylation of multiple sites occurs in the heart. To fully understand the molecular mechanisms underlying cardiac muscle regulation, Tn phosphorylation must be studied as integrated events. As an initial step towards understanding Tn crosstalk, we employed a two-model phosphomimetic approach using cardiac human Tn. We studied AMP-activated protein kinase (AMPK) TnI phosphorylation (TnI-S150D) and protein kinase C (PKC) cardiac troponin T (TnT) phosphorylation (TnT-T284E) to investigate the influence of these modifications on the effects exerted by TnI PKA phosphorylation (TnI-S23/24D). The combinatorial effects of these phosphorylation events on TnI-S23/24D was determined by measuring troponin C calcium binding properties in the reconstituted thin filament. Calcium dissociated from TnI-S23/24D thin filaments at 368/s compared to 103/s for wild-type (WT). Results demonstrate that TnI-S150D alone decreases calcium dissociation by 50%, while TnI-S150D integration with TnI-S23/24D blunted TnI-S23/24D kinetics by 15%, demonstrating the intramolecular crosstalk of TnI-S150D to alter the effects of TnI PKA phosphorylation. On the other hand, TnT-T284E calcium dissociation was identical to WT, but when present with TnI-S23/24D calcium dissociation was enhanced 136% compared to TnI-S23/24D alone. This demonstrates an intermolecular influence of TnT phosphorylation on TnI PKA effects. Overall, these findings demonstrate the combinatorial effects of Tn site-specific phosphorylation by AMPK and PKC crosstalk to affect the function of TnI PKA-induced phosphorylation through both intra- and inter- molecular mechanisms. These data further highlight the importance of understanding the role of integrated Tn PTM crosstalk on cardiac contractile regulation.

2822-Pos Board B592

Target Specific Phosphorylation of Cardiac Troponin I and Sex Dimorphic Myofilament Function in R403Q Mice

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Male mice expressing an autosomal dominant mutation in alpha-myosin heavy chain (R403Q) develop hypertrophic cardiomyopathy characterized by progressive left-ventricular dilation and cardiac dysfunction, whereas females do not. We hypothesize that this sexual dimorphism exists on multiple levels, including cellular metabolism regulation, post-translational modulation of contractile proteins and mechanical functions of contractile fibers. Hearts from wild-type (WT) and R403Q male and female animals with established disease were analyzed for myosin heavy chain (MyHC) isoform expression, total troponin I (TnI) phosphorylation, site-specific TnI phosphorylation, AMPKα expression, and AMPK activity. Ca2+-sensitive tension development was measured in demembranated cardiac trabeculae. R403Q male and female mice demonstrated progressive cardiac hypertrophy beginning at 4 months of age. β-MyHC expression increased in male and female R403Q mice. Total phosphorylation of TnI expression was independent of sex and R403Q mutation. No difference was found in phospho-TnI-Ser22/23 in R403Q animals relative to WT controls, however R403Q females had increased expression of phospho-TnI-Ser150 relative to WT counterparts. R403Q males showed increased expression of AMPKα, however had decreased AMPK activity relative to WT counterparts. R403Q females had reduced AMPKα expression but unchanged AMPK activity. The R403Q mutation does not affect Ca2+-sensitive tension development in demembranated cardiac trabeculae from males; female R403Q cardiac trabeculae were more sensitive to Ca2+ than WT controls. In conclusion, R403Q mice display hypertrophy with increased expression of hypertrophy marker β-MyHC. There exists a sexually dimorphic pattern of TnI